

## A record of a koinobiont endoparasitoid wasp, *Melalophacharops everese* (Hymenoptera, Ichneumonidae), attacking eggs of a lycaenid butterfly, *Acytolepis puspa*

Masami SHIMAZAKI<sup>1)</sup>\*, Kyohei WATANABE<sup>2)</sup> and Yoshiko SHIMAZAKI<sup>1)</sup>

<sup>1)</sup>440-114 Matsunami-cho, Takasago-cho, Takasago City, Hyogo, 676-0023 Japan

<sup>2)</sup>Laboratory of Insect Science, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe City, Hyogo, 657-8501 Japan

**Abstract** An ichneumonid wasp, *Melalophacharops everese* (Uchida, 1957) emerged from the final instar larvae of a lycaenid butterfly, *Acytolepis puspa* (Horsfield, 1828), that had been reared from the egg stage in captivity in the laboratory. The eggs, oviposited on a leaf of *Rosa multiflora*, had been collected in a field in Tanabe City, Wakayama Prefecture. Because the wasp could not have attacked the larvae in the laboratory, we believe that the eggs must have been parasitized before they were collected. The ovipositor of *M. everese* consists of a simple structure lacking the distinct dorsal subapical notch used for attacking active larvae. Although the biology of species with such an ovipositor is poorly known, this structure is strongly associated with hosts that are immobile. Therefore, we concluded that the parasitoid wasp had attacked eggs of *A. puspa*. This is the first report suggesting egg-larval development in *M. everese*.

**Key words** ovipositor shape, parasitoid host stage, ichneumonid wasp, Campopleginae.

### Introduction

The lycaenid butterfly, *Acytolepis puspa* (Horsfield) is found throughout Nansei Shoto and across Kyushu to southern parts of Shikoku and Honshu in Japan. It is common in certain localities and broods continuously. Its distribution is now expanding toward the north-east of Honshu. There are a few records of parasitism of *A. puspa* by the parasitoid wasp, *Melalophacharops everese* (Uchida) (Hymenoptera: Ichneumonidae), but the accounts are partly conflicting and the details have been poorly known. The parasitoid has been observed to emerge from (1) the reared prepupae (Kusigemati, 1967, 1988), (2) either the prepupae or the final instar larvae (Iwata, 1991), and (3) pupae reared from the first instar (Nakano, 1994). Nakano supposed that the larva had been attacked at the first instar stage, but did not provide any supporting evidence. *Melalophacharops everese* occurs in the Kyushu and Honshu areas of Japan and belongs to the subfamily Campopleginae, which are koinobiont endoparasitoid wasps. Six species of lycaenid butterflies have been reported as hosts in Japan (Kusigemati, 1988). However, at present there is no information available on when or how *M. everese* parasitizes its host species. Recently, a parasitoid wasp emerged from the final instar larva of *A. puspa* reared in our laboratory. Here, we identify the wasp as *M. everese* and suggest that it probably parasitizes the egg stage of *A. puspa*.

### Materials and Methods

Seven eggs of *A. puspa*, oviposited on leaves or stems of *Rosa multiflora* in a bush by the coastal road at Tenjinzaki in Tanabe City, Wakayama Prefecture, were collected on November 20, 2010. All of the eggs were kept in a lidded plastic container (Fig. 1, 20 × 14 × 5.5 cm). Hatched larvae were reared in the laboratory, where they were fed *R. multiflora* or *Myrica rubra* from November 2010 to February 2011. During rearing, six of the seven larvae showed apparent shrinkage in their body size and their body color changed from a shade of green to dark brown. Hereafter these larvae are described as “parasitized larvae”. Leaves with parasitized larvae were removed and transferred to another small lidded plastic box (Fig. 2, 10 × 7 × 3 cm) for observation. The remaining normal larva was fed *M. rubra* until it developed into an adult butterfly.

To identify the parasitoid wasp, we referred to the following descriptions and specimens: (1) the original description of *Horogenes everese* (=*Melalophacharops everese*) (Uchida, 1957), (2) a photograph of the holotype of *Horogenes everese* (data: Kumamoto City, Mt. Tatsuta, 1. xi. 1956, parasite of *Everes lacturnus*) taken by the second author in the Hokkaido University Museum, (3) the description of the female by Kusigemati (1967), and (4) a female specimen determined by Kusigemati (data: Riverside of Sagamigawa, Shake, Ebina City, Kanagawa Pref., 29. ix. 2002, I. Waki leg.).

\*Corresponding author: my.shima@win.email.ne.jp / my.shima@hotmail.co.jp

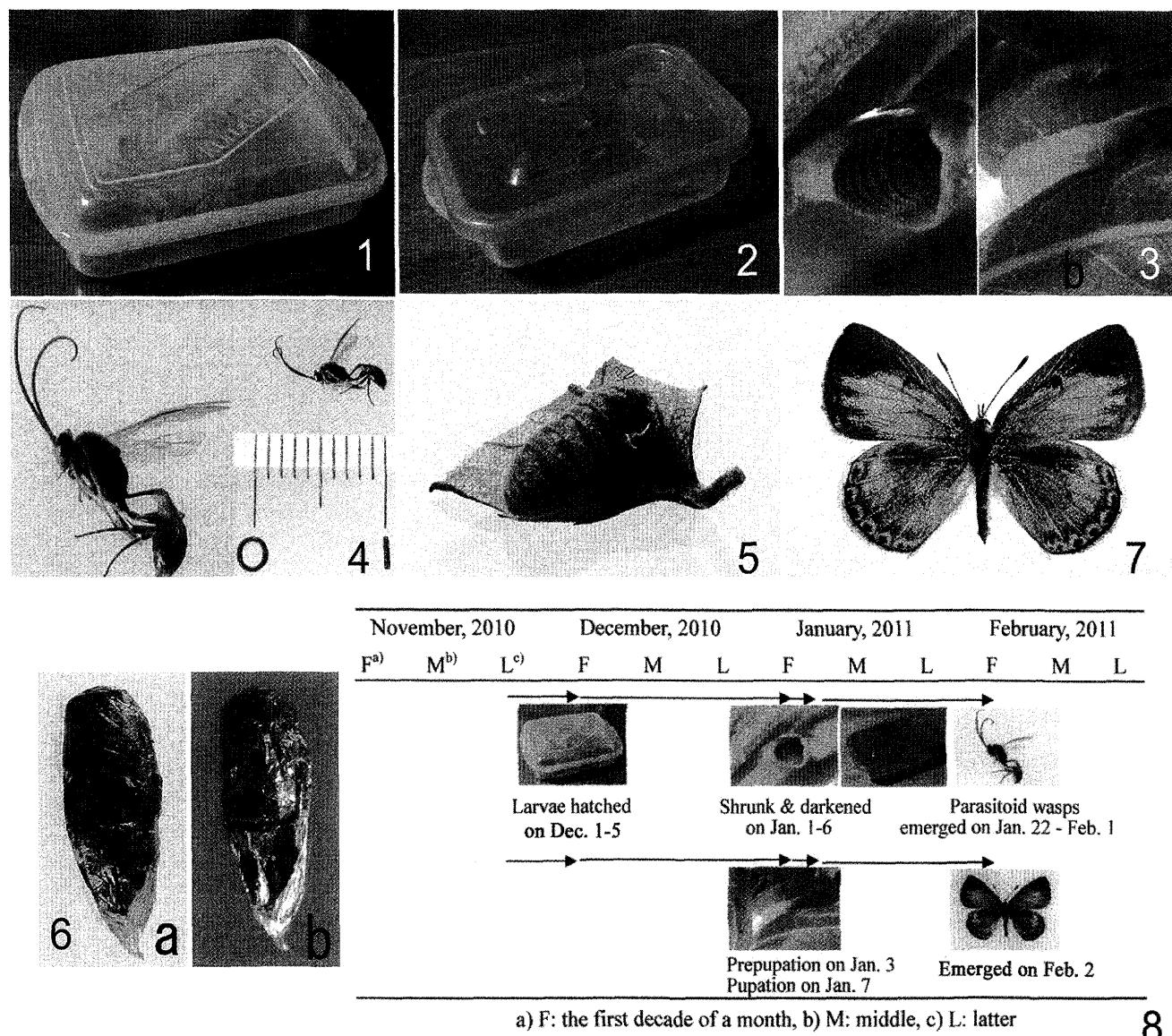


Fig. 1. Container used for rearing from egg stage.

Fig. 2. Box used for the parasitized larvae.

Fig. 3. *Acytolepis puspa*: a, parasitized final instar larva; b, normal prepupa.Fig. 4. Emerged parasitoid wasp *M. everese*.Fig. 5. The shell of the parasitized larva from which *M. everese* emerged.Fig. 6. Pupa of *M. everese* removed from the dead parasitized larva: a, dorsal aspect; b, ventral aspect.Fig. 7. Female *A. puspa*.

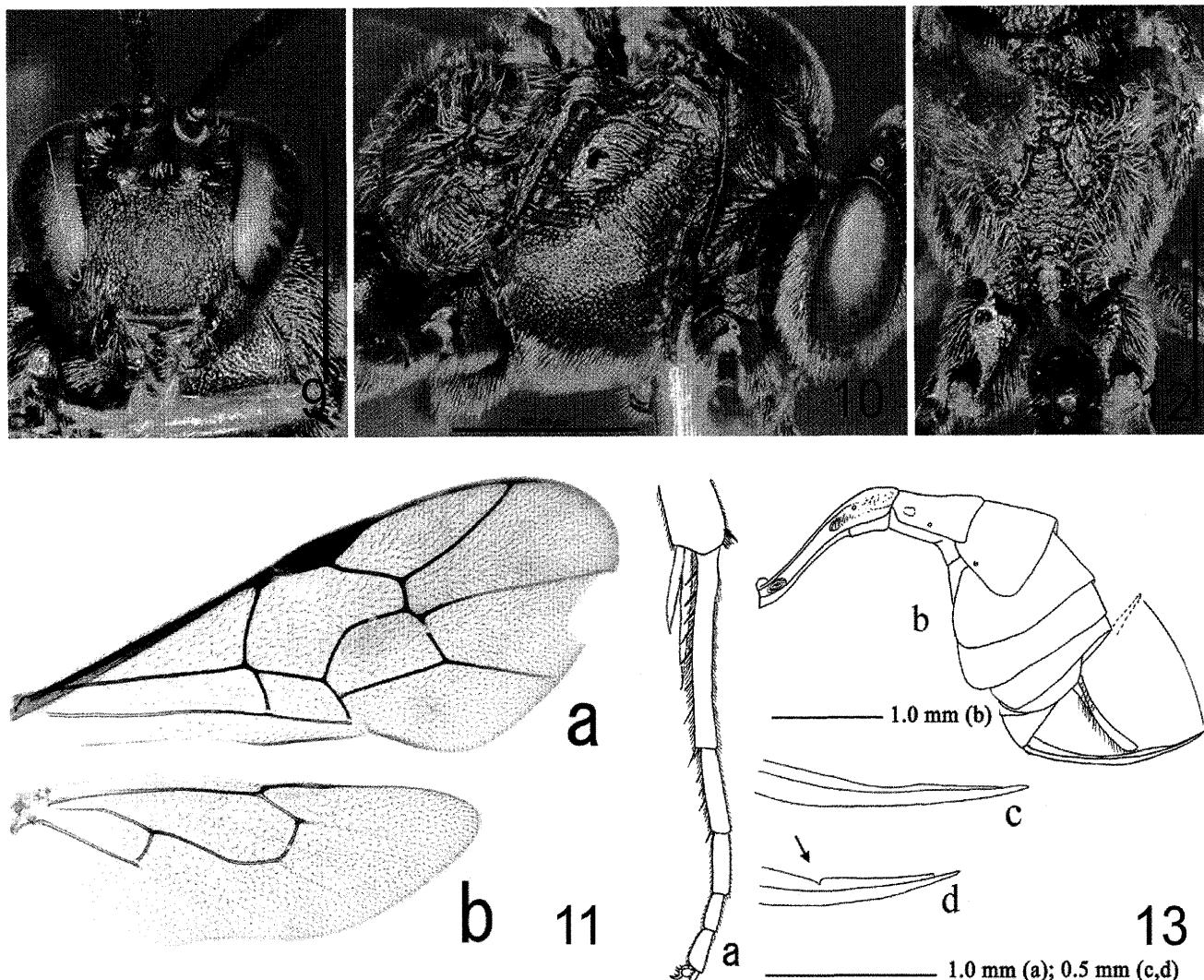
Fig. 8. Time course of rearing.

A stereomicroscope (SZ60, OLYMPUS Co., Tokyo, Japan; M165-C, LEICA CAMERA Co., Tokyo, Japan) and a light microscope (BX41, OLYMPUS Co.,) were used for observations and line drawings. Photographs of the specimen were taken with a digital microscope (VHX-1000, KEYENCE Co., Osaka, Japan). Digital images were edited using graphic software (Adobe Photoshop® CS3).

## Results

### 1. Hatching of eggs and abnormalities of the larvae

All eggs hatched in the rearing container between December 1 and 5, 2010. Two of the larvae were fed *R. multiflora* and the other five were given *M. rubra*. On January 1, 2011, a grown larva appeared to have settled on a leaf of *R. multiflora*, but its body color gradually changed from a shade of green to dark brown, and it shrunk in size

Fig. 9. Female of *M. everese*: head, anterior view. Scale bars 1.0 mm.Fig. 10. Female of *M. everese*: mesosoma, lateral view. Scale bars 1.0 mm.Fig. 11. Wings of *M. everese*: a, forewing; b, hindwing.Fig. 12. Female of *M. everese*: propodeum, dorsal view. Scale bars 1.0 mm.Fig. 13. Females of *M. everese* (a, b, c) and *Eriborus* sp. (d): a, hind tarsus, lateral view; b, metasoma, lateral view; c, d, apical part of ovipositor, lateral views. Arrow shows dorsal subapical notch.

(length: 6 mm; width: 3 mm). This abnormal change was later confirmed to be due to parasitism. Another parasitized larva settled on *R. multiflora* and similarly four larvae had settled on *M. rubra* (Fig. 3a) by January 6, 2011. We could not detect any silky girdle round the thorax, such as is usually spun by a normal prepupa, and all of the parasitized larvae remained on the leaves without shedding their skin during over the following three weeks. Then the larvae were transferred to the small lidded plastic box as described above and observations were continued. Only one larva, on leaves of *M. rubra*, grew normally to reach the prepupa stage (Fig. 3b, length: 10 mm; width: 5 mm) on January 3, 2011, and shed its skin to pupate on January 7, 2011.

## 2. Emergence of the ichneumonid parasitoid wasp from the final instar larvae

On January 22, 2011 a parasitoid wasp of the family Ichneumonidae (Fig. 4) emerged from the first parasitized larva, causing a sizable opening in the host (Fig. 5). Wasps of the same species emerged individually from another two parasitized larvae on January 24, 2011. To collect the specimens, we placed the box containing the wasps and remaining three parasitized larvae in a refrigerator. When one of the dead parasitized larvae was cut open, we could detect the dead pupa of *M. everese* (Fig. 6, length: 6 mm; width: 2.5 mm) inside the larva. A female *A. puspa* butterfly emerged from the unparasitized pupa on February 2, 2011.

(Fig. 7). The time course of the rearing is given in Fig. 8, which shows that the butterfly and the parasitoid wasps took similar times to develop and emerge.

### 3. Identification of the parasitoid wasp

The parasitoids were identified as *M. everese* based on the following characters: (1) thin and truncate anterior margin of clypeus (Fig. 9); (2) scutellum relatively strongly convex with apex acutely bent downward in lateral view; (3) upper half of mesopleuron covered with transverse rugae (Fig. 10); (4) forewing without areolet (Fig. 11a); (5) nervellus of hindwing without angulation (Fig. 11b); (6) propodeum covered with rugae and long silver hairs, areola and petiolar areas united (Fig. 12); (7) propodeal spiracle round; (8) first and second segments of hind tarsus with line of robust setae on ventral surface, at irregular intervals (Fig. 13a); (9) first metasomal tergite with glymma and subapical lateral shallow depression (Fig. 13b); (10) border between tergite and sternite of first metasomal segment located below the mid-height (Fig. 13b); (11) ovipositor short, its apex a little beyond apex of metasoma (Fig. 13b), without dorsal subapical notch (Fig. 13c); (12) body color black, second (female) or posterior part of second (male) and all following tergites reddish brown (Fig. 4); and (13) fore and mid legs yellowish brown excluding coxae, hind tibia, and basal part of hind basitarsus partly dull brown (Fig. 4).

## Discussion

Campopleginae are koinobiont endoparasitoid wasps, most of which attack larvae of Lepidoptera (Townes, 1970). Kusigemati (1967) was the first to report that the parasitoid wasp, *M. everese* emerged from a reared prepupa of *A. puspa*. Among previous reports, the observations by Iwata (1991) are almost similar to those presented here. In his report, two of five middle-instar larvae of *A. puspa* did not reach the prepupal stage and their body color changed to dark brown. Two *M. everese* females emerged from the darkened host larvae 12 days after the abnormal change. When he reared another middle-instar larva of *A. puspa*, the larva of the parasitoid wasp grew to pupate inside the host larva and a male *M. everese* emerged from the parasitized larva.

Taking these reports together, the wasp has been observed to emerge from three different stages of the host: the final instar larva, prepupa, and pupa. However, it is uncertain whether this is due to differing growth rate of each parasitoid larva or whether the attacking stage of the host was different. There has been a lack of information on when and how the host is parasitized. Here the koinobiont endoparasitoid wasp *M. everese* emerged from parasitized larvae that had

been reared from the egg stage in captivity. It is evident that the wasp could not have attacked the larvae in the laboratory. Thus we considered that the eggs of *A. puspa* had already been attacked by the parasitoid *M. everese* when we collected them from the field.

The time course of rearing (Fig. 8) suggests that a similar amount of time is required for the parasitoid wasp and the lycaenid larva to develop to adults. In addition, there is some observational evidence that the pupation of the larva of the parasitoid wasp was completed inside the parasitized larva although we could not know when pupation was complete.

The ovipositor of most species of Campopleginae and related subfamilies (e.g. Cremastinae, Banchinae and Ctenopelmatinae) has a dorsal subapical notch (e.g. another Campopleginae genus *Eriborus*, shown in Fig. 13d), and this structure may be used to provide some hold on the pierced integument of an active host larva. However, the ovipositor of *M. everese* lacks a dorsal subapical notch, and this condition suggests that it would favor attacking immovable eggs rather than mobile larvae during oviposition. A similarly un-notched ovipositor is shown in the Stilbopinae genus *Stilbops* (Townes, 1970), species of which are known to attack the egg stage of the host (Fitton, 1984; Shaw, 1989). Thus, we suppose that the absence of the notch on the ovipositor is more or less related to attacking the host at the egg stage. Whilst the usual mode of development in Campopleginae is larval-larval, there have been a couple of reports of egg-larval development. That with most detail is a recent study on *Hyposoter horticola* (Gravenhorst), where van Nouhuys and Ehmsten (2004) showed that the parasitoid oviposited in larvae shortly prior to their emergence from the egg.

The genus *Melalophacharops* comprises four described species in the Palearctic and the Oriental regions and three of these species are reported from Japan (Yu *et al.*, 2005). Although several species of lycaenid butterfly have been reported as host species for *M. everese*, the stage at which they are attacked is not demonstrated. To the best of our knowledge, this is the first report suggesting that the koinobiont endoparasitoid wasp, *M. everese* attacks the egg stage of its host, *A. puspa*.

## Acknowledgment

We would like to express our cordial thanks to Dr Gavin Broad (Department of Entomology, The Natural History Museum, London, UK) for his review of the manuscript and providing us with valuable suggestions.

## References

Fitton, M. G., 1984. A review of the British Collyriinae, Eucerotinae, Stilbopinae and Neorhacodinae (Hymenoptera: Ichneumonidae). *Entomol. Gaz.* **35**: 185–193.

Iwata, Y., 1991. Records on parasitoid insects for butterflies (1). *Gekkan Mushi* (243): 37–38. (In Japanese)

Kusigemati, K., 1967. New Host Records of Ichneumonidae from Japan. *Ins. matsum.* **29** (2): 50.

Kusigemati, K., 1988. Parasitoid wasp Ichneumonidae for Japanese butterflies. *Satsuma*. **37** (100): 149–160. (In Japanese)

Nakano, J., 1994. Parasitoid wasp for *Acytolepis puspa*. *Choken Field* **9** (7): 23. (In Japanese)

Shaw, M. R., 1989. A host record of *Stilbops limneriaeformis* Schmiedeknecht (Hymenoptera: Ichneumonidae, Stilbopinae) in Scotland. *Entomol. Gaz.* **40**: 5–6.

Townes, H. K., 1970. The genera of Ichneumonidae, part 3. *Mem. Amer. Ent. Inst.* **13**: 1–307.

Uchida, T., 1957. Drei aus den Schmetterlingslarven gezuechtete Ichneumonidenarten. *Ins. matsum.* **21**: 59–61.

van Nouhuys, S. and J. Ehrnsten, 2004. Wasp behavior leads to uniform parasitism of a host available only a few hours per year. *Behav. Ecol.* **15**: 661–665.

Yu, D. S., K. van Achterberg and K. Horstmann, 2005. World Ichneumonoidea 2004. Taxonomy, biology, morphology and distribution. [CD/DVD computer program]. Taxapad®, Vancouver, Canada.

## 摘要

シジミチョウの内部寄生蜂ツバメシジミチビアメバチ *Melalophacharops everese* (Uchida, 1957) によるヤクシマ

ルリシジミ卵への寄生例 (島崎正美・渡辺恭平・島崎能子)

和歌山県田辺市天神崎海岸沿い道路傍の野生ノイバラに産卵されていたヤクシマルリシジミの野生卵7個を採取し、最初から密閉容器内で飼育した。孵化した幼虫2個体はノイバラ、5個体にはヤマモモの新葉を与えて成育させた結果、ノイバラ飼育の1個体に、前蛹化直前の終令幼虫段階で著しい体長の縮みと体色の黒褐色化を認めた。引き続きノイバラ飼育1個体とヤマモモ飼育4個体が同様の状態を示したため、これら全6個体を小さな密閉容器へと移して観察を続けていたところ、約3か月後に最初の個体から寄生蜂が羽化脱出した。引き続き同じ寄生蜂2個体が発生した段階で、まだ寄生蜂が出ない個体共々冷凍庫で殺虫処理をして寄生蜂を標本にした。本種はその形態観察から、すでにヤクシマルリシジミなど6種のシジミチョウへの寄生記録が知られる飼い殺し型内部寄生蜂ツバメシジミチビアメバチ *Melalophacharops everese* (Uchida, 1957) だと同定できた。今回の採集飼育条件から、ツバメシジミチビアメバチはヤクシマルリシジミの卵に産卵したと考えられる。ツバメシジミチビアメバチと同じ亜科や近縁亜科の多くの種は寄主幼虫に産卵し、その産卵管の先端方背面に切れ込みがあるのに対し、本種は産卵管に切れ込みを欠くので、この産卵管形態は寄主卵に産卵することと関連していると考えた。この寄生蜂がシジミチョウ成育過程のいずれの段階で寄生攻撃をするのかはこれまで知られておらず、今回の“ヤクシマルリシジミの卵が寄生されていた”という知見は初めての記録だと思われる。

(Received March 15, 2011. Accepted August 26, 2011)